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PHARMACOLOGICAL STUDIES OF THE OPTIC SYSTEM OF THE CHICK: EFFECT OF γ-AMINOBUTYRIC ACID AND PENTOBARBITAL*

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Abstract—The optic system of the young chick was selected for the present studies because endogenous γABA levels can be elevated in the optic tectum by parenteral administration of solutions of this compound. The results obtained have shown that intravenously administered γABA can alter the electrical activity of the optic system at both the retinal and tectal levels. The action of γABA upon the electroretinogram (ERG) which has not been previously described in any species, modified the electrical activity of the eye in a manner seemingly different from that reported for other substances tested in various species On the basis of the present results, it is suggested that γABA acts as an inhibitor of neural control of retinal activity.

The effect of pentobarbital was also studied. Its effect upon the behavior of the avian optic system is similar to that observed for this drug in the mammalian cortex and on the ERG of a variety of species, including man. The effect of pentobarbital upon the ERG, correlated with anatomical features of the avian optic system, suggests an action mediated through centrifugal nerves, possibly the isthmo-optic tract which arises from a caudal midbrain nucleus—the isthmo-opticus. The data further seem to support the view that this centrifugal system is inhibitory and may be associated with adaptation to the dark.

Results of these experiments with pentobarbital and γABA alone or in combination, together with the similarity of action of pentobarbital upon the electrical activity of the avian tectum and mammalian cortex, led to the suggestion that γABA exerts its action in the avian tectum at the presynaptic level.

These studies indicate that the optic system of the chick can serve as a useful and subtle tool for neuropharmacological studies in general.

SINCE the isolation of γ-aminobutyric acid† from the central nervous system, a great deal of attention has been directed toward an analysis of its mechanism of action.[1] It has been difficult to assess the physiological significance of this substance in the intact vertebrate CNS. Although decreases and increases in the levels of γABA can be produced in the CNS by the administration of several substances, the observed physiological changes cannot be attributed solely to changes in γABA because all such

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† The following abbreviations are used throughout the text: γABA, gamma-aminobutyric acid; CNS, central nervous system; ERG, electroretinogram; TSA, spontaneous electrical potentials recorded from the optic tectum; PER, electrical response evoked in the tectum by photic stimulation of the retina; NR, electrical response evoked in the optic nerve by photic stimulation of the retina.

substances may have multiple effects. Elevation of the γABA content in the normal CNS by administration of γABA, itself, has not been feasible because γABA does not appear to pass the blood barrier in the species ordinarily employed for such studies. However, it has been found that γABA parenterally administered to the young chick during the first two weeks after hatching can pass into various areas of the CNS, including the optic lobe.[2] In the young chick the electrical activity of the brain showed essentially mature patterns by the first week after hatching.[3-6] Thus in the chick it seemed possible to study the effects of parenterally administered γABA on a relatively stable responding system rather than on one undergoing rapid developmental changes.

The entry of exogenous γABA into the optic lobe and the favourable morphological characteristics of the avian optic system suggested that this system would be particularly suited for the study of the action of γABA and, possibly, other neurotropic substances. The optic nerve undergoes a complete decussation and continues in an uninterrupted fashion to synapse in the superficial strata of the optic tectum.[7] The entry of the optic innervation at the tectal surface facilitates recording a potential that can be evoked by photic stimulation of the retina, which should have pre- and postsynaptic components, thus making this system potentially suitable for the analysis of the site of effect of drugs that influence central synaptic activity. Since retinal elements are involved, additional evaluation of drug action at morphologically similar but functionally different synaptic sites seemed possible.

The purpose of the present study was to develop and standardize techniques for the study of the effect of γABA as well as other centrally active substances on the optic system of the young chick. This report describes these techniques and the effects of γABA and pentobarbital.

METHODS

Rhode Island Red chicks of both sexes at 7 to 14 days after hatching, weighing 63 to 114 g, were used as experimental subjects.

Preparation

Preparation consisted of tracheal intubation with polyethylene tubing and cannulation of the ischiadic vein and artery under ethyl chloride and ether anesthesia, observing the special precautions required for avian species.[8-10] The anesthetized animal was placed in a specially designed holder, the head was immobilized by screws placed in the external auditory meati, and the entire unit then fitted into a stereotaxic instrument (Trent Wells, South Gate, Calif.). The portion of the unit holding the body of the chick incorporates a heating element energized by a standard 6-v storage battery. Body temperature of the animal was maintained at 38° by means of a thermistor probe placed in the rectum. The control unit for the probe (Yellow Springs Instruments, model 71) served as an on–off switching device for the battery input.

The dorsal surface of the tectum was exposed by removing the overlying calvarium. Occasionally, for studies of drug effects on the electroretinogram (ERG), the optic nerve was surgically interrupted. In these cases the nerve was exposed by retraction of the frontal lobe of the cerebrum and excision of the orbital roof.

Prior to recording, pressure points and all operative sites were treated with a local anesthetic (Cetacain), general anesthesia was discontinued, and the preparation was

further immobilized by intravenous administration of 0·6 mg of tubocurarine*, with subsequent doses given as required. Adequate respiratory exchange was provided by means of a Harvard variable rate–volume respirator. Two hours were allowed for recovery from the general anesthetic prior to recording.

Recording

The characteristically high cardiac rate of the young chick (300 to 350 beats/min) provided an excellent monitor of the general physiological state and was recorded throughout the preparatory and experimental periods by means of external electrodes differentially coupled through a Grass P–5 amplifier to an audioamplifier and speaker and to a paper recorder. Blood pressure was recorded from the ischiadic artery with a Statham transducer and coupler.

The sites from which CNS recordings were made are illustrated diagrammatically in Fig. 1. Monopolar recordings of tectal spontaneous activity (TSA) and photically

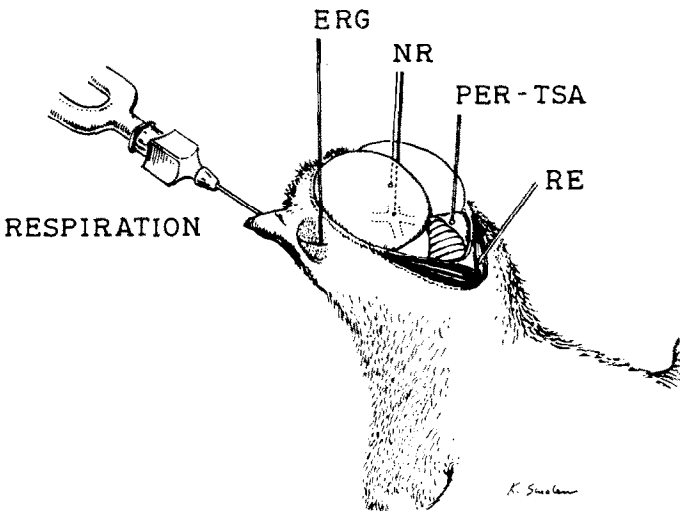

FIG. 1. Diagram of the head and exposed brain of a young chick depicting electrode placements for recording electrical activity from the optic system. ERG, electroretinogram; PER, photically evoked response recorded from the tectum; TSA, tectal spontaneous activity recorded between stimulations; NR, nerve response to photic stimulation; RE, reference electrode inserted into the neck muscles.

evoked responses (PER) were obtained from the dorsolateral aspect of the tectal surface with a gross chlorided-silver ball electrode against a reference electrode that had been thrust into the dorsal neck muscle mass near the occipital insertion. The ERG was obtained by placing the critical electrode on the temporal border of the cornea (limbus corneae). In some experiments, optic nerve activity (NR) was recorded through insulated stainless steel electrodes inserted dorsoventrally through the cerebrum into the chiasma or visually inserted into the intraorbital portion of the exposed nerve. The signals obtained were differentially coupled through Tektronix type 122

* Six tenths mg of *d*-tubocurarine is 10 to 20 times the dose required to produce a paralysis of 10 to 20 min duration in the young chick.[38] This dosage level was selected because it extends the duration of paralysis to about 1 hr per injection, it is suitably tolerated by the animal, and it causes no observable effect upon the parameters being studied.

low-level preamplifiers (time constant of 1 sec) to a Tektronix 502 dual-beam oscilloscope and photographed with a Grass Kymograph camera. All signals were simultaneously monitored on a paper recorder.

Stimulation

Evoked activity was obtained by photic activation of one eye. The stimulus was provided by a Grass PS–2 photostimulation unit with the strobe lamp placed 20 in from the chick's eye, parallel with the optic axis. Unless otherwise noted, each experiment was preceded by 30 to 45 min of dark adaption. The frequency of stimulation never exceeded 1/30 sec. Intensities are according to the settings of the Grass stimulator at positions 1, 4, and 16. According to the manufacturer, relative intensities at these settings are 1, 16, and 80 times (corresponding to 10 log values of 0·0, 12·0, 19·0).

Drugs

The drug administration was by intravenous infusion through the ischiadic vein by means of a Harvard constant-infusion pump, at 0·079 ml/min. This method of administration was necessary to minimize the effects upon the cardiovascular system of the drugs used, both of which are potent hypotensive agents when given more rapidly. At dose levels at which maximal effects are obtained, pentobarbital causes a 10 per cent decrease in diastolic pressure despite these precautions (see Results). One disadvantage of the slow-infusion technique is that there may be greater metabolic and excretory removal of the drug tested than with a single rapidly administered dose, particularly in the chick, which has a high metabolic rate. The criterion for discontinuation of the drug was the attainment of a maximal response change. For these reasons the amount of drug required to produce the effects described in this study are not necessarily comparable to dose–response data obtained by others using different techniques.

The γABA solution (100 mg/ml) was prepared immediately prior to use by dissolving the crystalline material in normal (0·9 %) saline. In one series of experiments γABA was applied dropwise onto the tectal surface as a 10 % solution prewarmed to 37°. Solutions of pentobarbital sodium (Nembutal, 6 mg/ml) were prepared by dilution of the commercial preparation in normal saline. Since the latter preparation contained alcohol (10 %) and propylene glycol (20 %) as additives, control experiments were conducted in which normal saline containing the same concentrations of alcohol and glycol was infused in amounts up to twice those given in the pentobarbital studies. No detectable effects were observed.

RESULTS

γ-Aminobutyric acid

(1) *Intravenous infusion.* γABA, parenterally administered to an unanesthetized, curarized, and dark-adapted young chick, produced characteristic effects upon the PER and TSA. These effects, illustrated in Fig. 2, are reversible and reproducible within the same preparation and have been remarkably consistent in more than 150 separate experiments. They consist of a block of the PER and the NR and a profound alteration in the ERG. The degree of the last effect is markedly dependent upon the stimulus strength, changing from an apparent inhibition at the lowest intensity (Fig. 2B, top) to a marked enhancement of both amplitude and duration at the highest (Fig. 2B, bottom). The degree of enhancement, while involving both the negative (a-wave) potential and positive (b-wave) potential, is far greater on the latter than on the

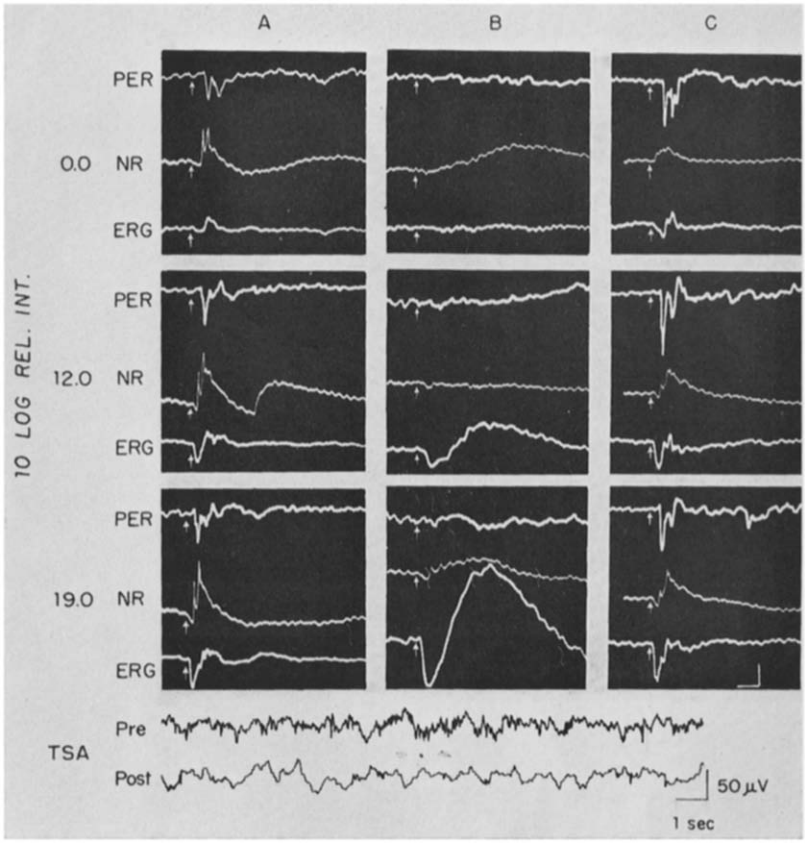

FIG. 2. The effects of intravenous infusion of γABA upon the evoked and spontaneous activities recorded from the optic system of the young chick, at three different stimulus intensities (ordinate). A, Control responses; B, the same parameters during maximal drug effect; C, 3 hr after B. Extended traces at bottom of figure illustrate drug effects on the spontaneous rhythm recorded from the tectum. TSA, Pre: spontaneous activity preceding drug administration; TSA, post: the same during maximal drug effect. In this and all other figures the arrows denote point of stimulation; downward deflections of the traces denote negativity at the critical electrode; calibration mark (⌊) = 50 msec and 100 μV.

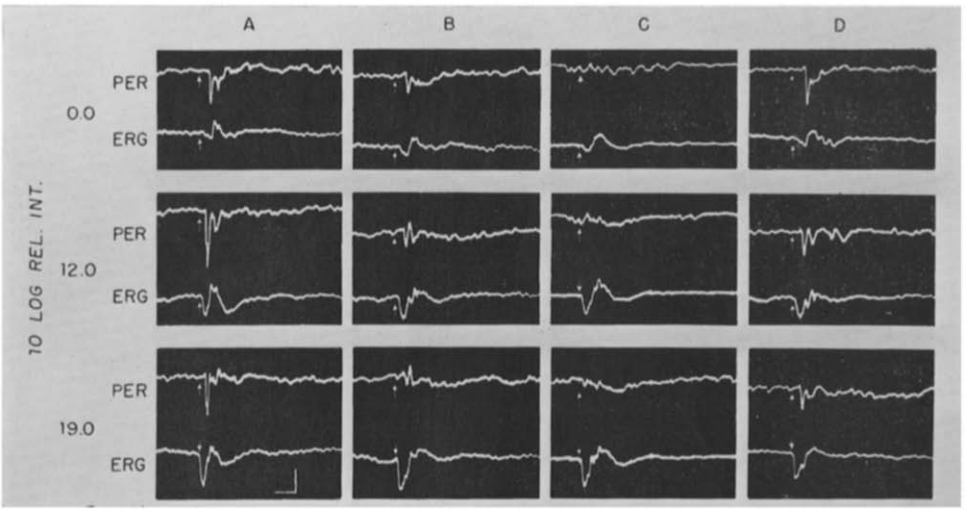

FIG. 3. The effect of γABA on the photically evoked response (PER) and electroretinogram (ERG) when applied topically to the tectal surface. A, Control; B, 30 min after beginning the application; C, 60 min after A; D, 2 hr after rinsing the tectal surface with warm (37 °) normal saline.

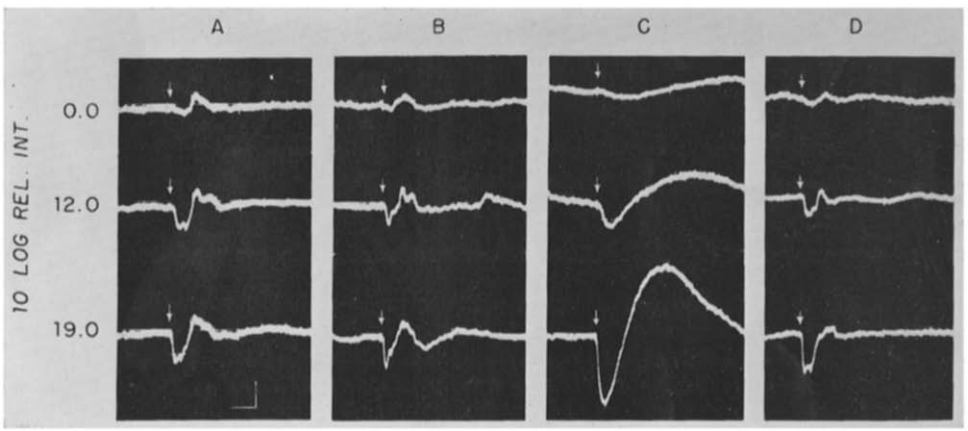

FIG. 4. The effect of intravenously administered γABA on the electroretinogram after surgical interruption of the optic nerve. A, ERG with optic nerve intact, after 30-min dark adaptation; B, ERG response after section of the optic nerve; C, during maximal effect of γABA; D, 1 hr after drug.

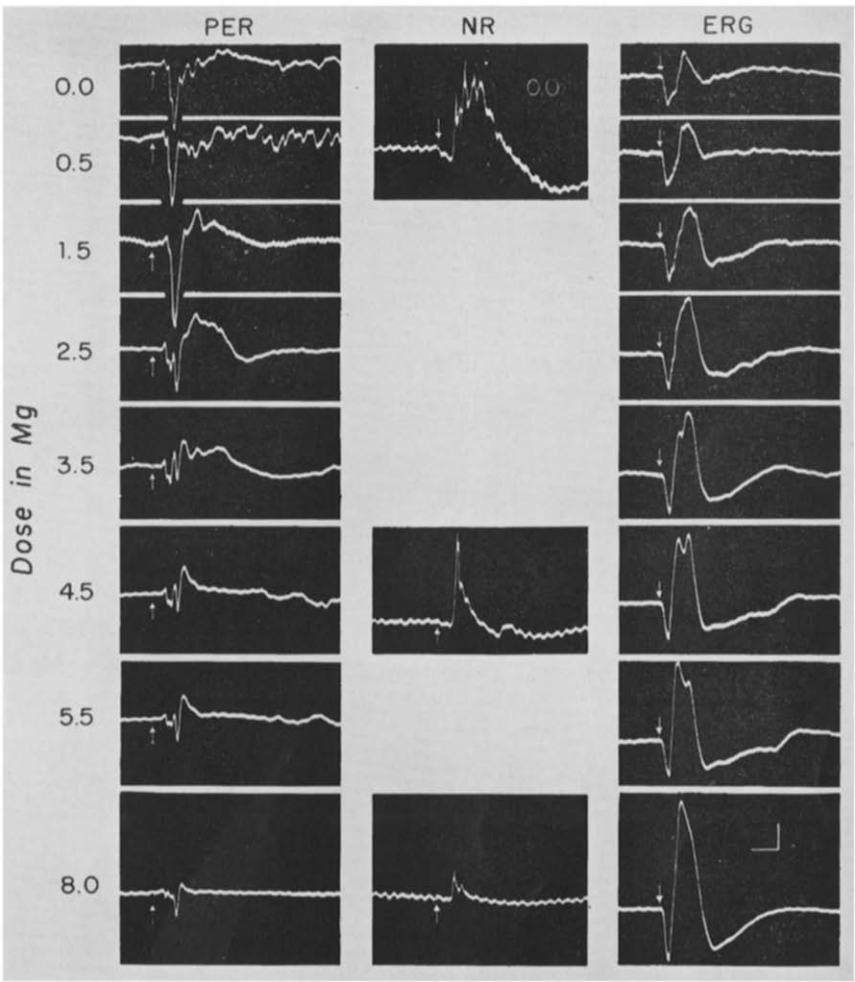

FIG. 5. The dose-response effects of intravenously administered pentobarbital upon the recorded potentials from the young chick optic system. PER, NR, ERG, and time-amplitude marker same as in preceding figures. Note double b-wave (ERG column, 3·5 to 5·5 mg level). Note also the failure of pentobarbital to completely block PER, at highest dose levels. Dosages are absolute values. The record shown was from a 14-day-old animal weighing 120 g.

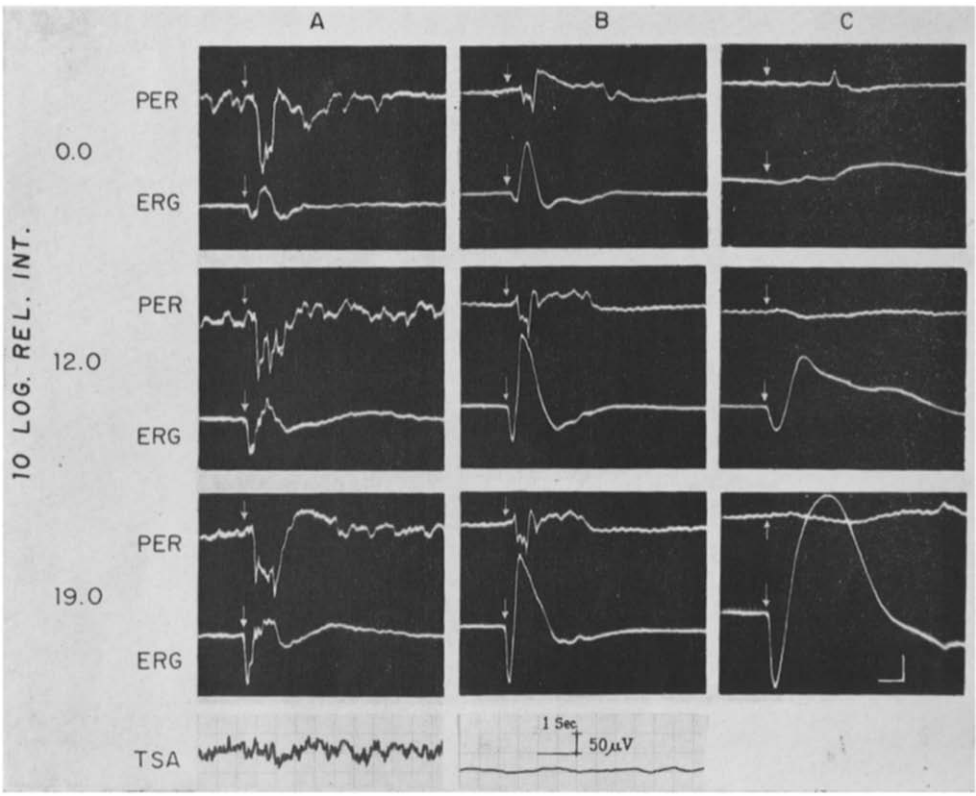

FIG. 6. The effect of intravenous infusion of γABA on the PER, ERG, and TSA during maximal pentobarbital action. A, Control; B, maximal pentobarbital effect; C, maximal γABA infused immediately after recording of B. At bottom (TSA) are pen-recorder traces of tectal spontaneous activity before and during pentobarbital effect. Responses in this and Fig. 7 have been retouched to facilitate reproduction.

former component. The effect of γABA on the TSA was inconstant. Either no change was observed or the type of record shown at the bottom of Fig. 2 (TSA post) was obtained.

The amount of γABA required to produce maximal response change ranged between 0·8 and 1·3 g/kg body weight. Although such doses appear to be high, the technique of administration (see Methods) and the biochemical data[2] show that probably less than 0·5 per cent of the total amount injected is found in the brain at any time. Recovery of the recorded parameters of the optic system occurred within 1·5 and 3 hr (Fig. 2C), with recovery of the ERG preceding that of the PER by 1 to 1·5 hr.

Typically, although the NR did not attain predrug levels (Fig. 2C), the PER appeared to be greater than during the control period at all stimulus intensities studied. This suggests the possibility that during action of γABA compensatory mechanisms may be brought into play which persist for a period sufficiently long that when the γABA levels are decreasing toward normal an increased responsivity may be observed at the tectal level. This phenomenon is currently under further study.

(2) *Local application of γABA to the optic tectum.* In no instance did topical application of γABA to the exposed tectal surface produce any changes in the ERG or NR, and the TSA remained largely unaffected, However, the PER was inhibited (Fig. 3C). Maximal effect occurred only after 30 to 60 min and required repeated application of the drug. The dura was intact in all cases. Topically applied γABA unmasked a chiefly positive PER during the onset of action (Fig. 3B) which was finally depressed when maximal effect* was attained (Fig. 3C). In the case of parenterally administered γABA no PER positivity was noted. Recovery from topical application took place between 1 and 2 hr after rinsing the tectal surface with normal saline (Fig. 3D). During recovery the positivity of the PER did not reappear.

(3) *The effect of intravenous γABA on the ERG after section of the optic nerve.* It was observed that the ERG recovered from γABA earlier than did the PER (see above). Furthermore, the loss of the NR occurred concomitantly with the maximal change in ERG. These findings suggested that the changes in the ERG might be attributable to a direct action of γABA at the retinal level. Experiments were performed to test this possibility. Figure 4 shows a typical experiment demonstrating the ERG response to intravenously administered γABA after surgical interruption of the optic nerve. In all cases the effect of γABA upon the ERG was indistinguishable from that which occurred with the nerve intact (cf. Fig. 2B and 4C). Recovery from γABA occurred normally (Fig. 4D). Because the normal ERG was not changed greatly by section of the optic nerve (Fig. 4B), it was necessary to confirm completeness of nerve section. This was accomplished by infusion of pentobarbital after recovery from γABA (see below) and by post-mortem dissection.

Pentobarbital sodium

(1) *Intravenous infusion.* It was of interest to compare the effects of γABA with those of another CNS-depressant drug, the central actions of which are better understood. Pentobarbital was chosen for study because it is generally accepted that

* Maximal effect for both i.v. and topical γABA was considered to have been obtained when the amplitude of the evoked response was reduced below that of the spontaneous rhythm (TSA).

barbiturates exert a central effect through an action upon synaptic transmission;[11–14] possibly, as more recent evidence indicates, by a selective action at the postsynaptic level.[15]

The changes in the recorded parameters induced during the intravenous infusion of pentobarbital are illustrated in Fig. 5.

(2) *Electroretinogram.* Figure 5 (right column) shows a series of ERGs recorded at maximal stimulus strength as a function of the drug dosage. The control response is indicated by 0-mg level. For each animal a maximal effect was observed beyond which additional drug produced no further change. During the infusion a progressive increase of both amplitude and duration of the b-wave occurred. There was also a slight increase in the amplitude of the a-wave at the highest dose and a decrease in its duration. In contrast to the marked prolongation observed with γABA, the overall duration of the ERG was less altered by pentobarbital.

During pentobarbital infusion a small potential appeared on the leading edge of the b-wave (3·5-mg level, Fig. 5) separating it into two components. As the drug dosage was increased, this potential gradually progressed through the summit of the wave to the trailing edge and finally disappeared as the maximal drug effect was achieved.

The effect of pentobarbital on the ERG at different stimulus intensities can be seen in Fig. 6B. At low stimulus intensities the control ERG (Fig. 6A, top) was small. Pentobarbital increased this response, the relative enhancement being greater at higher stimulus intensities. On the other hand, γABA appeared to inhibit the response at low stimulus intensity (Fig. 2B and 6C, top).

(3) *The effect of pentobarbital after section of the optic nerve.* Surgical section of the optic nerve rendered the ERG almost entirely insensitive to the pentobarbital effects, in agreement with the findings of Jacobson and Gestring[16] in cat and monkey. Occasionally the b-wave showed a slight increase at maximal stimulus intensities, but such changes were not striking compared to the usual pentobarbital effect. This is in marked contrast to the findings reported above for γABA.

(4) *Effects of pentobarbital on PER, NR, and TSA.* The effects of pentobarbital on the TSA (bottom of Fig. 6) consisted initially of a flattening or elimination of all activity. As the drug infusion continued, a depression of the PER and NR occurred until only approximately 25 per cent of the initial response remained (Fig. 5, PER, NR). These responses did not change when additional drug was administered or when the stimulus frequency was increased. When the dose was increased to fatal levels they disappeared within 30 sec after cessation of heart beat. The doses of pentobarbital required to produce observable effects ranged between 15 and 25 mg/kg for minimal effects and between 55 and 80 mg/kg for maximal. The lower doses correspond to those that induced light to moderate surgical anesthesia. The higher doses are in the range at which cardiovascular depression is difficult to avoid.

The configuration of the residual PER during maximal pentobarbital effect consisted primarily of a negative wave preceded by a small positive potential upon which one or more smaller potentials were imposed (Fig. 5, 6, and 7). Measurement of the latencies of these smaller potentials, including the initial positive wave, show that they coincide with the latencies of the spikes of the control NR (Fig. 5, top). They may, therefore, be a reflection of optic nerve activity at the tectal level, and the negative wave is thought to be a reflection of presynaptic electrogenesis (see Discussion).

Administration of γABA during maximal pentobarbital effects

The striking difference in the effects of these substances upon the electrical activities of the avian optic system as described above suggested that different mechanisms might be involved in their respective actions. Independence of action might be made evident if a clear cut, characteristic effect could be obtained when one of these substances was administered during the maximal effect of the other. Such interrelationship of action is shown in Fig. 6 and 7.

In addition to the effects observed on the electrical activities as depicted, it is of interest to note that there were no additive effects of maximal doses of both substances upon the peripheral system as judged from the blood pressure, heart rate, EKG, and body temperature. This observation seems to lend additional support to the possibility of the independent modes of action of γABA and pentobarbital.

The ERG response during the simultaneous maximal effect of both drugs is shown in Fig. 6, 7, and 8. In all cases the ERG was chiefly typical of a γABA response. A

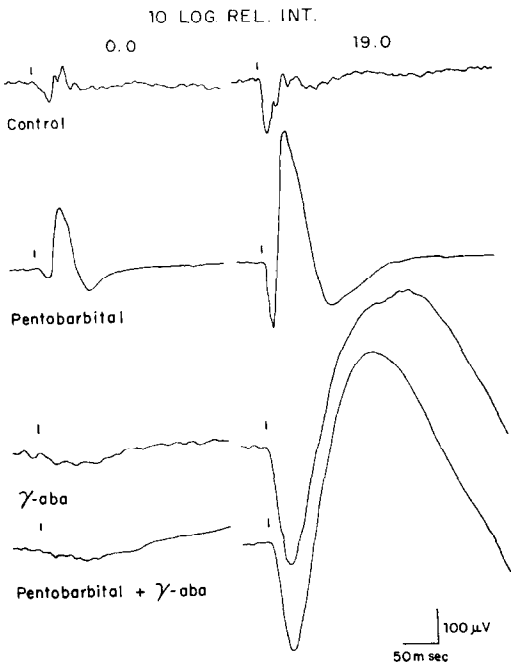

FIG. 8. Summary of the action of γABA and pentobarbital on the electroretinogram (ERG) of the chick, and the response change as a function of photic intensity. The tracings on the left were recorded at the lowest intensity used; those on the right at the highest intensity used. Top pair shows control ERG after 30-min dark adaptation, second pair after pentobarbital, third pair after γABA, and bottom pair the effect of γABA during maximal effect of pentobarbital.

small but consistent difference in the ERG recorded during the action of both drugs was noted in that there was an increase in amplitude and decrease in duration of the b-wave as compared to the typical γABA response. The significance of this latter effect is not known. However, it does not appear to be the result of an algebraic summation of the γABA and pentobarbital responses. For the present, γABA appears capable of affecting the ERG in its usual manner, even when the ERG has already been altered

by the action of pentobarbital. Identical results were obtained when γABA infusion preceded that of pentobarbital.

The effect of γABA infusion on that portion of the PER which persisted during maximal pentobarbital action is shown in Fig. 7. The primary negative wave was found to be more sensitive to γABA action and was depressed first, whereas the smaller wavelets remained unchanged. When γABA effect was maximal, as determined by the ERG change, inhibition of the small potentials also occurred. A similar sequence of changes on the PER during maximal pentobarbital effect was observed during asphyxia.

DISCUSSION

The intravenous infusion of γABA and pentobarbital produced distinctly different effects upon the electrical activity recorded from the optic system of the young chick. Both substances markedly altered the photically evoked potential recorded from the tectum (PER) and the electroretinogram (ERG), whereas only pentobarbital consistently affected the tectal spontaneous activity (TSA).

Electroretinogram

The comparitive effects of both drugs upon the ERG at low and high stimulus intensities are summarized diagrammatically in Fig. 8.

(1) *Pentobarbital.* The action of pentobarbital upon the avian ERG (Fig. 5, 6B, 8) appears strikingly similar to that observed in the rat [17] and in the cat and monkey [16, 18] and suggest that similar mechanisms are involved.

The existence of centrifugal fibers in the mammalian (including man) and avian optic nerve which terminate in the retina seems fairly certain,[16,19,20] but their origins and functions remain largely unknown. The lack of pentobarbital effect after section of the optic nerve in cat and monkey was interpreted by Jacobson and Gestring[16] as additional evidence for the existence of centrifugal fibers in mammals. They postulated a center in the brain which controls retinal function by "regulating the rate and/or the intensity of retinal activity and controlling dark adaption." The present results provide similar evidence for the existence of centrifugal influences in the chick and seem to allow additional inferences as to their origin and function.

The avian optic system and its connections, features of which have been clearly summarized by Ariens Kappers *et al.*,[7] include a group of fibers within the optic tract termed the medial optic tract or *tractus isthmo-opticus*. This group of fibers is related to a nuclear mass, the *nucleus isthmo-opticus*, located within the mesencephalon, and recent anatomical evidence [21,22] shows that it has a centrifugal course. The relatively large dose of pentobarbital required to produce the effects observed in our experiments suggest that its site of action is on an area of low anesthetic sensitivity, conceivably within the mesencephalon; thus it possibly affects the above system of centrifugal retinal control.

The double b-wave component, which is clearly evidenced during pentobarbital infusion (Fig. 5), is also of interest. Similar b-waves have been observed in man, monkey,[23] and chick[24] under various conditions of stimulation. These waves seem to be characteristic of all vertebrates with rod-and-cone retinas, and have usually been attributed to the separate activities of the rods and cones. Crescitelli[25] has shown that this is not unequivocally true. He observed a double b-wave in the ERG of the antelope

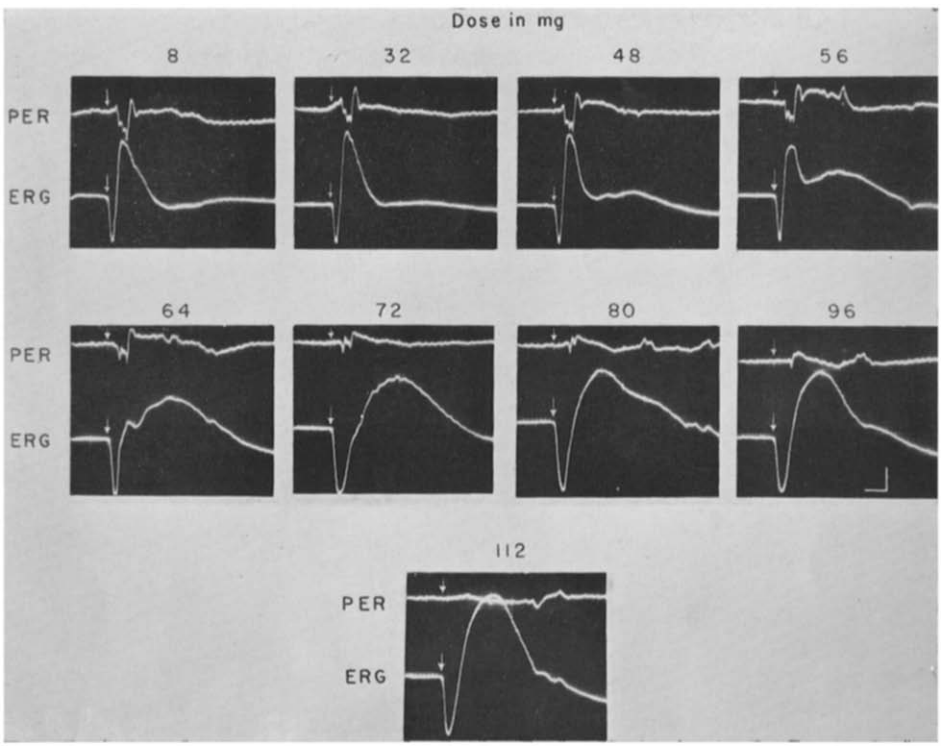

FIG. 7. The effect of increasing doses of γABA infused during maximal pentobarbital action. Doses are absolute values. Record shown was from a 12-day-old animal weighing 100 g. Note that during the infusion there occurs first a reduction of the amplitude of the negative wave of the PER preceding depression of the small potentials imposed upon it (see text).

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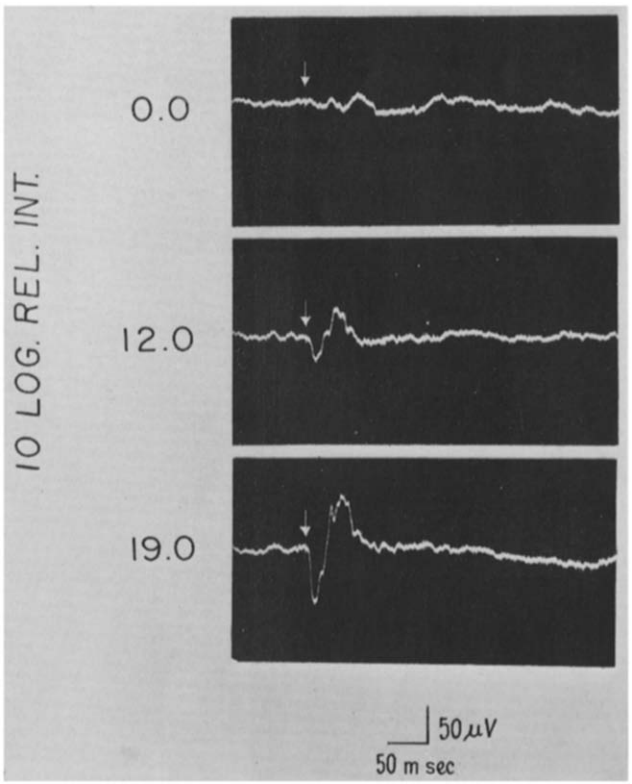

FIG. 9. Control record of the electroretiongram of a young chick taken under conditions of normal room illumination. Note similarity of the configuration of the bottom trace seen here to the dark-adapted pentobarbitalized ERG (Fig. 5).

ground squirrel, an animal with a pure cone retina. There are some similarities in the ERG recorded from the dark-adapted eye of the antelope ground squirrel (Fig. 1, Ref. 25) and from the pentobarbitalized, dark-adapted eye of the chick, which are of interest. These similarities possibly may be attributable to the fact that the studies on the ground squirrel were conducted with the animal under pentobarbital anesthesia. These data suggest that under a variety of conditions the double b-wave in the ERG, observed in vertebrates with both mixed and pure retinas, might be attributable to some other feature of the optic system that these species have in common. Since it has been shown in the present study that pentobarbital can cause the appearance of a double b-wave and that its action is most probably through a centrifugal mechanism, it is suggested that the feature common to all the species studied is the presence of cephaloretinal influences.

Additional data which may also support the view that the cephaloretinal system plays a role in dark-adaption[16] is provided by our finding that the ERG recorded from the light-adapted chick eye has an enhanced, two-component b-wave (Fig. 9, bottom), which appears similar in configuration to the ERG of the dark-adapted chick anesthetized with pentobarbital.

(2) *γABA*. There is a considerable literature describing the modification of the electrical activity of the retina resulting from the administration of a variety of substances.[16,18,26–31] However, the effects of γABA upon the ERG, which to our knowledge have not been previously described in any species, appear to be significantly different, at least in the chick, from those of the other substances studied.

The effect of γABA on the ERG was markedly dependent upon stimulus intensity. At low intensity an apparent inhibition occurred, while at higher intensities the ERG was enhanced greatly in duration and amplitude as compared to the control (Fig. 2B, 8). In contrast with the ERG, the activity within the optic nerve was inhibited by γABA at all stimulus strengths. It seemed evident, therefore, that a functional block of retinal–optic nerve transmission had occurred rather than just an increase in the threshold of retinal sensitivity. The direct action of γABA at the retinal level, as shown by the persistence of its characteristic effects after section of the optic nerve (Fig. 3), and previous alterations in the ERG as a result of pentobarbital action (Fig. 6, 7, 8), would seem to locate the blockade of the nerve response at the optic nerve ganglion or peripheral to it.

Since γABA is capable of affecting the cardiovascular system,[32] it is possible that one mechanism by which its action could be induced would be through an effect upon the retinal vasculature.[18,26,27] To our knowledge it has not yet been determined whether vasodilator drugs are capable of inhibiting the transmission of retinal activity to the optic nerve. However, it can also be shown that pentobarbital produces effects upon the cardiovascular system of the chick similar to those induced by γABA (observed in our laboratory), and it would thus seem that their dissimilarity of action upon the avian ERG would rule out a dominant role of a vascular effect as a mechanism of action of γABA. Further evidence in support of this view is the fact that drugs such as hexamethonium[16] and acetylcholine,[18] which also reduce blood pressure, produce an opposite effect to γABA, a reduction in the amplitude of the ERG.

These data lead us to believe that the action of γABA under our experimental conditions results in a loss of neuronal control of retinal activity and that the recorded

ERG might be a reflection of the generalized, uncontrolled discharge of the specialized retinal photoreceptive units.

Photically evoked response and tectal spontaneous activity

The action of pentobarbital upon recorded responses of the avian tectum resembles its effects upon the activities recorded from the mammalian cortex and requires little further discussion. This similarity is of importance, however, since some features of pentobarbital action may provide a basis for an interpretation of the mode of action of γABA within the vertebrate brain.

In the present studies the small tectal potential, which remained during maximal pentobarbital effect (Fig. 5A, 6B, 7), was not appreciably affected by an increased rate of stimulation, by increased intensity (Fig. 6B), or by increased doses of the drug. This residual potential seems to be analogous to the "primary" cortical response[13] or the "initial spike"[14] recorded from the primary sensory cortex of the cat during sciatic stimulation. It was suggested that the source of these potentials was associated with the arrival of excitation at the cortex, possibly reflecting events in the axon terminals. A similar suggestion was made (see Results) for the source of the pentobarbitalized PER from the avian tectum, especially because the synapses between the optic nerve and neurons of the tectum occur at the tectal surface, the site from which the present recordings were obtained. The disappearance of this residual PER after γABA administration (Fig. 7) would therefore seem consistent with the suggestion that γABA inhibits at the presynaptic level. The failure of γABA, when given alone, to suppress the TSA while inhibiting the PER could also be explained in a similar way and still could be consistent with the view that spontaneous electrical activity of the brain is chiefly a manifestation of postsynaptic dendritic potentials,[33] but then poses the question of how these potentials are generated.

Although not in complete agreement with the description of the effects of iontophoretically applied γABA on most synapses in the spinal cord of the cat,[34] the above suggestion appears to be consistent with most of the current literature concerning the action of γABA in the vertebrate nervous system.[35,36] Indeed, exogenously applied γABA has been shown to inhibit the excitatory neuron at the presynaptic ending at the neuromuscular junction of the crayfish, presumably by decreasing the release of excitatory transmitter.[37]

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